CYSTEINE THIOL-PROTECTED PEPTIDES FOR USE IN IMMUNOASSAYS

Field of the Invention

The subject invention relates to peptide compositions which mimic epitopes for use in immunoassays and, more particularly, to improved peptide compositions having enhanced epitope presentation and enhanced specific immunoreactivity with antibodies produced in response to native proteins.

Background of the Invention

Currently individuals with antibodies reactive with human immunodeficiency virus (HIV) are determined by immunoassays of the conventional sandwich ELISA format. These assays are comprised of an immobilized viral lysate that is contacted with blood or serum suspected of containing HIV antibodies. While the existing commercial tests appear to have significantly diminished the transmission of HIV via blood products, viral lysate-based tests have several significant disadvantages. These disadvantages include, but are not limited to, the need to grow and handle large quantities of live infectious virus; the possibility that live virus might be incorporated into test materials; the variable nature of the resulting lysate; and the substantial number of false positive and false negative results which necessitate additional combinatory testing, such as by Western blot.

In commonly owned US Patent 4,629,783 to Cosand, incorporated herein by reference, several synthetic peptides are disclosed which mimic immunologically reactive viral proteins epitopes. Among them, a peptide comprising a sequence of 26 amino acids of gp41, designated peptide 39, was found to be reactive

with all known HIV-1 positive sera tested and was not
reactive with any negative sample tested. Subsequently,
several groups have recognized the importance of the
peptide 39 region defined by Cosand as containing a
dominant antigenic determinant. See Wang, J.J.G. et al.
Proc. Natl. Acad. Sci. 83:6159 (1986), Shoeman, R.L. et
al. Analyt. Biochem. 161:370 (1987), Gnann, J.W. et al.
J. Virol. 61:2639 (1987), and Gnann, J.W. et al. J.
Infect. Dis. 156:261 (1987).

Synthetic peptides offer significant advantages
over viral lysates. Advantages include, but are not
limited to, specificity, purity, ease of production, etc.
Potential disadvantages include the need to use more than
one peptide to detect all viral strains; and peptides
being inherently small in size, loss of immunological
reactivity upon immobilization on a solid phase.

Small peptides used as immobilized antigens in
immunological assays are generally synthesized by solid
phase methods, wherein each amino acid which comprises
the peptide is added sequentially in a protected form
while the growing peptide chain is immobilized on an
insoluble resin support. During the synthetic process
side chains of certain amino acids comprising the growing
chain are protected, blocked by a variety of chemical
groups which remain until the completed peptide is
removed from the insoluble resin support, usually by
reaction with concentrated hydrofluoric or trifluroacetic
acid. During cleavage the majority of the protective
groups are removed and the completed peptide is purified
by any of a variety of methods well known in the art.

Crude or purified peptide is then immobilized
onto a solid phase by a variety of methods, including
direct conjugation, conjugation to a soluble non-
immunologically reactive protein or other inert molecule,
or by direct adsorption onto a solid phase. US Patent
4,629,783 discloses examples of several of these methods.

Peptides which mimic epitopes from the gp36 protein of HIV-2, a glycoprotein believed to be comparable in retroviral function to gp41 of HIV-1, are disclosed in co-owned US patent applications USSN 030,403 and USSN 035,408, filed March 25, 1987 and April 7, 1987, respectively, herein incorporated by reference. Among these, a peptide designated 41-2-1, comprised of 26 amino acids encoded within a region similar to that of peptide 39 in HIV-1, was found to be reactive with all known HIV-2 positive sera tested and was not reactive above appropriate cut-off levels with negative samples which were tested. A shortened version of peptide 41-2-1, designated 41-2-3, comprising amino acids residues from the carboxyl end of peptide 41-2-1, was also found to be reactive with the HIV-2 sera and in some cases performed better than peptide 41-2-1.

Peptides 39, 41-2-1 and 41-2-3 each contain within their amino acid sequence two cysteine residues. The presence of cysteine residues within a peptide sequence allows for the formation of inter- and intra-molecular disulfide bonds during purification, immobilization, and upon long term storage of a deprotected peptide. Therefore, such peptide compositions are usually immobilized on a solid phase as a mixture of a variety of oxidative forms, including monomer, dimer and polymers of various sizes. Precautions are generally not taken to control the oxidative form of peptides immobilized on a solid phase. Rosen, J.I. et al. (WO87/06005) recognized that certain peptides derived from the highly antigenic region of HIV-1, identified by Cosand, supra, which contained more than one cysteine residue were immobilized as a mixture of various oxidative forms and suggested that the oxidative form of a peptide, particularly polymers, may be of importance to the reactivity of certain peptides. Rosen et al. disclose that in some cases the cyclic form of certain peptides is less efficient at binding to solid

surfaces than polymeric forms. The variety of oxidative forms of the peptides may be a source of variability in sensitive immunoassay and this may also influence the results based on those assays.

Accordingly, there exists a significant need in the art for peptide compositions with improved reactivity, and which provide as few false-negative and false-positive results as possible. The present invention fulfills these and other needs.

Summary of the Invention

Improved peptide compositions useful in the detection of HIV viruses or antibody to HIV viruses are provided. Methods are also provided for the preparation of improved peptide compositions which allow for control of the oxidative form of peptides comprising said composition prior to and following immobilization on a solid phase as an antigen in immunoassay methods. Control of the oxidative form provides for peptide compositions with improved immunological reactivity as measured by specificity and sensitivity. Peptides are synthesized using standard solid phase peptide synthesis methods with the exception that a chemically reversible protection means resistant to the highly acidic cleavage conditions is substituted for commonly used S-benzyl blocking of cysteine thiol groups. Chemically reversible protection may be include, for example, ethylcarbamoyl, acetamidomethyl, 3-nitro-2-pyridinesulfinyl, diphenyl-4-pyridyl-methyl, and the like.

An alternate embodiment exists when reversible protection of cysteine thiol groups is provided after the assembly of the amino acid sequence. Reduction of the peptide composition prior to protection of the peptide is necessary to insure no disulfide bonds remain prior to protection. Also, cysteine thiol protection may be removed prior to immobilization wherein deprotection and

oxidation of the peptide are carried out in solution prior to immobilization of the oxidized peptide composition to a solid phase. Conditions for oxidation are selected such that the formation of intra-molecular disulfide bonding is favored, i.e., very dilute and neutral to weakly alkaline conditions.

In certain embodiments of the invention the peptides generally are comprised of the amino acid sequence:

(I)

Y-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-
Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys*-
Ser-Gly-Lys-Leu-Ile-Cys*-X;

(II)

Y-Arg-Val-Thr-Ala-Ile-Glu-Lys-Tyr-Leu-Gln-Asp-
Gln-Ala-Arg-Leu-Asn-Ser-Trp-Gly-Cys*-
Ala-Phe-Arg-Gln-Val-Cys*-X; or

(III)

Y-Gln-Asp-Gln-Ala-Arg-Leu-Asn-Ser-Trp-Gly-
Cys*-Ala-Phe-Arg-Gln-Val-Cys*-X

wherein X is OH or NH_2, Y comprises additional amino acids added to enhance the reactivity of the peptides and Cys* is a cysteine residue comprised of a thiol group protected by chemically reversible means. A particularly preferred embodiment is where Y is the sequence Cys-Gly-Gly.

A wide variety of immunoassay protocols may use peptide compositions, which peptides may be in solution or immobilized on a solid phase. Various solid phases may be used including latexes (microparticulates) of

silica, cellulose, fluorocarbon polymers, polyacrylamide
and polystyrene. Solid phases comprising, e.g., silica,
cellulose, fluorocarbon polymers, nylon, polyacrylamide
and polystyrene may also be used. Immobilization to the
solid phase may be either by adsorption or by covalent
bonding.

One immunoassay protocol disclosed in the
present invention for determining the presence of
antibodies to HIV viruses or antigens of HIV viruses in a
body fluid, generally comprise the steps of (a)
contacting, under conditions which permit immobilization,
a solid phase and a composition of at least one peptide
comprising the amino acid sequence:

(I)

Y-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-
Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys*-
Ser-Gly-Lys-Leu-Ile-Cys*-X;

(II)

Y-Arg-Val-Thr-Ala-Ile-Glu-Lys-Tyr-Leu-Gln-Asp-
Gln-Ala-Arg-Leu-Asn-Ser-Trp-Gly-Cys*-
Ala-Phe-Arg-Gln-Val-Cys*-X; or

(III)

Y-Gln-Asp-Gln-Ala-Arg-Leu-Asn-Ser-Trp-Gly-
Cys*-Ala-Phe-Arg-Gln-Val-Cys*-X

wherein X is OH or NH_2, Y comprises additional amino acids
added to enhance the reactivity of the peptides and
Cys* is a cysteine residue comprising a thiol group
protected by chemically reversible means; (b) removing
the chemically reversible protection means from the
cysteine thiol groups of the immobilized peptide; (c)

incubating the immobilized peptide under conditions
conducive to disulfide bond formation; (d) contacting
under conditions which permit immunospecific binding a
body fluid with the immobilized peptide to form a
reaction mixture; (e) detecting whether immunospecific
binding has occurred between the immobilized peptide and
an antibody component of the body fluid in which the
detection of immunospecific binding indicates the
presence of antibodies to the HIV viruses in the body
fluid.

A second immunoassay format comprises the steps
of (a) removing the chemically reversible protection of
the cysteine thiol groups; (b) incubating the deprotected
peptide under conditions conducive to intra-molecular
disulfide bond formation to form a peptide composition;
(c) immobilizing the peptide composition on a solid
phase; (d) contacting under conditions which permit
immunospecific binding a body fluid with the immobilized
peptide composition to form a reaction mixture; and (e)
detecting whether immunospecific binding has occurred
between the immobilized peptide composition and an
antibody component of the body fluid in which the
detection of immunospecific binding indicates the
presence of antibodies to the HIV viruses in the body
fluid.

Immunospecific binding may be detected by
removing unbound components from reaction products formed
in the immunoreaction mixture, adding a labeled antibody
to the immunoreaction mixture, the labeled antibody being
capable of immunospecifically binding to a component of
the reaction product and the label providing a detectable
signal. Unbound components may be removed from the
reaction mixture by filtration.

Other aspects of the invention will become
evident upon reference to the following detailed
description and the appended claims.

IV. DESCRIPTION OF THE SPECIFIC EMBODIMENTS

The present invention relates to improved peptide compositions for use in immunoassay methods for the quantitation and/or detection of antibodies or antigens. In certain embodiments the peptides are used in the detection of viral antigens or antibodies thereto, and more particularly the antigens or antibodies associated with retroviruses, such as HIV-1 and HIV-2 and the related family of lentiviruses. Other exemplary peptides prepared in accordance with the present invention mimic epitopic regions of HTLV-I and HTLV-II.

Peptides may be identified which are capable of binding to antibodies elicited by a protein which is introduced to a foreign host. In several instances Cys residues comprise a portion of the peptide epitope(s) which recognizes and binds the antibodies. Protection of the Cys residues during the synthesis of the peptides hinders the development of oxidative forms of the peptides and provides improved peptide immunological reactivity, as measured by, e.g., increased sensitivity and/or specificity.

Peptide compositions of the subject invention have cysteine residues within said sequence chemically modified so as to be reversibly protected from reaction, e.g., oxidation. Thus a peptide useful in the present invention will generally have at least two Cys residues which contribute to the conformation of the peptide capable of binding antibodies engendered by the native protein. Generally at least two of the Cys residues will be capable of forming intra-molecular disulfide bonds. To form the intra-molecular bonds the Cys residues are separated by at least one amino acid residue, typically at least two, and more usually from about three up to about 15 or more residues. In certain preferred embodiments the Cys residues are separated by about five

to eight residues. By forming intra-chain disulfide bonds the vicinal Cys residues present a loop, or cyclic peptide region which may be important in enhanced immunoreactivity.

Peptides of the subject invention may be prepared in a wide variety of ways. The peptides generally range from about six up to about 50 amino acid residues, more typically from about six up to about 35 amino acids. Because of their relatively short length they may be synthesized in solution or on a solid support in accordance with conventional techniques. See, for example, Stewart and Young, Solid Phase Peptide Synthesis, 2nd edition, Pierce Chemical Company, 1984; and Tam et al., 1983, J. Am. Chem. Soc. 105:6442, which are incorporated herein by reference.

Alternatively, hybrid DNA technology may be employed for expression of the desired peptide in transformed eukaryotic or prokaryotic host cells. See, for example, Maniatis et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, N.Y. 1982, incorporated herein by reference. Of course, it should be understood that by peptide is meant a sequence of at least about six amino acids, typically ten or more, up to 200 amino acids or more, including entire proteins. The expressed peptide may then be isolated and protected as herein described.

Reversible chemical protection of the cysteine thiol side chain of the subject peptides may be carried out during the assembly of the peptide sequence by chemical synthesis of the peptide composition. When a subject peptide composition is assembled by standard solid phase techniques, a chemically reversible protection means is selected for resistance to the conditions used during the amino acid addition cycles and to the high concentrations of acid used to cleave the peptide from the resin solid phase. Among the several thiol-protecting groups available, the choice for a

particular synthesis will likely depend on the structure
of the peptide being synthesized and the nature of other
protecting or blocking groups being synthesized, as well
as the particular reagent chosen for cleavage of the
peptide from the resin. Protection means suitable for
use in the present invention include, for example, but
are not limited to, acetamidomethyl (Stewart and Young,
supra), 3-nitro-2-pyridinesulfinyl (Matsueda, et al.
1986, Int. J. Peptide Res. 28:167, Ridge, R.J. et al.
1986, Int. J. Peptide Res. 19:490), and diphenyl-4-
pyridylmethyl (Coyle, S. et al., 1976, J. Chem. Soc.
Chem. Comm. pg. 980). A particularly preferred
protection is S-(N-ethylcarbamoyl). See, St. Gutmann
(Helv. Chim Acta. (1966) 49:83) and Storey H. T. et al.
(J. Amer. Chem. Soc. (1972) 94:6170). Each of the
foregoing articles is incorporated herein by reference.

Alternatively, the cysteine thiol groups may be
reversibly protected after assembly of the amino acid
sequence. In this embodiment the peptide, which may be
purified or otherwise enriched in cyclic or polymeric
forms, is reduced prior to protection of the peptide to
ensure few if any disulfide bonds remain.

The peptides which are thiol-protected are
deprotected for use in an immunoassay. The peptide may
be used in solution, either free or bound to a carrier,
or adsorbed to a solid phase. Cysteine thiol protection
may be removed prior to immobilization, if the peptide is
to be attached to a solid phase, wherein deprotection and
oxidation of the peptide are carried out in solution.
Means for deprotection will correspond to a large extent
to the particular protecting group used and are well
known by those skilled in the arts. Alternatively,
deprotection and oxidation may be performed during or
subsequent to immobilization. Conditions for oxidation
are selected such that the formation of intra-molecular
disulfide bonding is favored, i.e., very dilute and
neutral to weakly alkaline conditions.

For the detection and/or quantitation of antibodies to HIV, particularly HIV-1, an exemplary peptide capable of mimicking the HIV-1 glycoprotein, gp41, includes the following peptide sequence, where oligopeptides of at least about seven amino acids and less than about 50 will be included within the sequence:

(I)

Y-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-
Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys*-
Ser-Gly-Lys-Leu-Ile-Cys*-X;

wherein X is OH or NH_2, Y comprises optionally present amino acids added to enhance the reactivity of the peptides and Cys* is a cysteine residue comprising a thiol group protected by chemically reversible means.

For the detection and/or quantitation of antibodies to HIV-2, exemplary peptides capable of mimicking the HIV-2 glycoprotein, gp36, includes the following peptide sequences, where oligopeptides of at least about seven amino acids and less than about 50 will be included within the sequence:

(II)

Y-Arg-Val-Thr-Ala-Ile-Glu-Lys-Tyr-Leu-Gln-Asp-
Gln-Ala-Arg-Leu-Asn-Ser-Trp-Gly-Cys*-
Ala-Phe-Arg-Gln-Val-Cys*-X; or

(III)

Y-Gln-Asp-Gln-Ala-Arg-Leu-Asn-Ser-Trp-Gly-Cys*-
Ala-Phe-Arg-Gln-Val-Cys*-X

wherein X is OH or NH_2, Y comprises optionally present amino acids added to enhance the reactivity of the peptides and Cys* is a cysteine residue comprising a thiol group protected by chemically reversible means.

For the detection and/or quantitation of antibodies to HTLV-I and HTLV-II, exemplary peptides

capable of mimicking the HTLV-I and -II transmembrane
glycoproteins, both designated gp21, include the
following peptide sequences, where oligopeptides of at
least about seven amino acids and less than about 50 will
be included within the sequences:

IV   (HTLV-I)
Y-Gln-Asn-Arg-Arg-Gly-Leu-Asp-Leu-Leu-Phe-Trp-
Glu-Gln-Gly-Gly-Leu-Cys*-Lys-Ala-Leu-Gln-Glu-
Gln-Cys*-X; and

V (HTLV-II)
Y-Gln-Asn-Arg-Arg-Gly-Leu-Asp-Leu-Leu-Phe-Trp-
Glu-Gln-Gly-Gly-Leu-Cys*-Lys-Ala-Ile-Gln-Glu-
Gln-Cys*-X;

wherein X is OH or NH_2, Y comprises optionally present
amino acids added to enhance the reactivity of the
peptides and Cys* is a cysteine residue comprising a
thiol group protected by chemically reversible means.

It should be understood that the peptide
compositions of the present invention need not be
identical to any particular protein or retroviral
sequence, such as an HIV polypeptide sequence, so long as
the subject compositions are able to mimic an epitope and
thus provide for immunological competition to some extent
with the native protein, e.g., gp41 glycoprotein of at
least one strain of HIV-1 or the native gp36 glycoprotein
of at least one strain of HIV-2.

For example, the peptides of interest may be
modified by introducing conservative or nonconservative
substitutions in the peptides, usually fewer than 20
number percent, more usually fewer than 10 number percent
of the amino acids being exchanged. It may be desirable
to vary one or more particular amino acids to more
effectively mimic the differing epitopes of the native

protein, such as the different retroviral strains, for example.

Therefore, the subject polypeptides may be subject to various changes, such as insertions, deletions and substitutions either conservative or nonconservative where such changes might provide for certain advantages in their use. By conservative substitutions is intended combinations such as gly, ala; val, ile, leu; asp, glu; asn, gln; ser, thr; lys, arg; and phe, tyr. Usually, the sequence will not differ by more than 20% from the sequence of at least one strain on HIV except where additional amino acids may be added to either terminus to provide for an "arm" by which the subject peptides may be, for example, conveniently immobilized.

In addition, the peptide sequence may differ from the natural sequence in the modification of the terminal NH_2 by acylation, e.g., acetylation, or thioglycolic acid amidation, terminal carboxy amidation, e.g., ammonia, methylamine etc. In some instances, these modifications may provide sites for linking to a support or other molecule.

As mentioned above, amino acid arms may be provided at the C- and/or N-terminus of the peptide or oligopeptide. If present, the arms will usually be at least one amino acid and may be 50 or more amino acids, more often 1 to 10 amino acids, and preferably less than 5 amino acids for ease of synthesis. The arms may serve a variety of purposes, such as spacers, to attach peptides to a carrier, to immobilize peptides to a solid phase, etc. To provide useful functionalities for linking to a carrier, solid phase or to form higher-ordered structures, such as dimers, trimers, or other multimers, amino acids such as tyrosine, cysteine, aspartic acid, or the like, may be introduced at provided at the C- and/or N-terminus of the arm or peptide. To enhance epitope presentation, of particular interest is the presence of from 1 to 10 amino acids at the C- and/or

N-terminus, more preferably 1 to 5 amino acids, and most
preferably about 1 to 3. Particularly preferred
embodiments of certain peptides described herein are
obtained when 3 amino acids are added as an arm,
generally at the N-terminus, with the N-terminal residue
of the arm preferably Cys. In exemplary embodiments the
spacer residues between the peptide and the terminal
functional group are Gly. Thus a particularly preferred
peptide has an arm comprising Cys-Gly-Gly, where the Cys
is the N- or C-terminal residue. A terminal Cys residue
may also be linked through a disulfide linkage to a
dithio- or thio-functionalized support or a thioether
linkage to an activated olefin support.

Peptide sequences of particular interest for
detecting antibodies to HIV-1 are derived from the
sequence of Peptide 39 as defined by Cosand, supra.
Peptide 39GC, is comprised of the 26 amino acids of
Peptide 39, above, wherein Y is comprised of the sequence
Cys-Gly-Gly- and X is comprised of -NH$_2$. The peptide
therefore is comprised of the amino acid sequence:

Peptide 39GC
Cys-Gly-Gly-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-
Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys*-
Ser-Gly-Lys-Leu-Ile-Cys*-NH$_2$

wherein Cys* are cysteine residues protected by
chemically reversible means.

Similarly, peptide sequences of particular
interest for detecting antibodies to HIV-2 are derived
from the sequence of Peptide 41-2-1, wherein Y is
comprised of the sequence Cys-Gly-Gly- and X is comprised
of -NH$_2$. The peptide therefore is represented by the
amino acid sequence:

Peptide 41-2-1GC

Cys-Gly-Gly-Arg-Val-Thr-Ala-Ile-Glu-Lys-Tyr-Leu-Gln-

                Asp-Gln-Ala-Arg-Leu-Asn-Ser-Trp-Gly-Cys*-
                    Ala-Phe-Arg-Gln-Val-Cys*-NH$_2$

wherein Cys* are cysteine residues protected by
chemically reversible means.

        Peptide 41-2-3GC, is comprised of the sequence
of 41-2-3, above, wherein Y is the sequence Cys-Gly-Gly
and X is NH$_2$ and is represented by the amino acid
sequence:

                    Peptide 41-2-3GC

Cys-Gly-Gly-Gln-Asp-Gln-Ala-Arg-Leu-Asn-Ser-Trp-Gly-Cys*-
                Ala-Phe-Arg-Gln-Val-Cys*-NH$_2$

wherein Cys* are cysteine residues protected by
chemically reversible means.

        Peptide sequences of particular interest for
detecting antibodies to HTLV-I are derived from the gp21
sequence of Peptide IV. In a preferred embodiment,
peptide 121-1GC1, Y is comprised of the sequence Cys-Gly-
Gly- and X is comprised of -NH$_2$. The peptide therefore is
represented by the amino acid sequence:

                        121-1GC1
        Cys-Gly-Gly-Gln-Asn-Arg-Arg-Gly-Leu-Asp-Leu-
        Leu-Phe-Trp-Glu-Gln-Gly-Gly-Leu-Cys*-Lys-Ala-
                Leu-Gln-Glu-Gln-Cys*-NH$_2$,

wherein Cys* are cysteine residues protected by
chemically reversible means.

        Peptide sequences of particular interest for
detecting antibodies to HTLV-II are derived from the gp21
sequence of Peptide V. In a preferred embodiment,
peptide 221-1GC1, Y is comprised of the sequence Cys-Gly-
Gly- and X is comprised of -NH$_2$. The peptide therefore is
represented by the amino acid sequence:

                        221-1GC1

Cys-Gly-Gly-Gln-Asn-Arg-Arg-Gly-Leu-Asp-Leu-
Leu-Phe-Trp-Glu-Gln-Gly-Gly-Leu-Cys*-Lys-Ala-
Ile-Gln-Glu-Gln-Cys*-NH$_2$;

wherein Cys* are cysteine residues protected by chemically reversible means. It should be understood that due to substantial sequence homology between the HTLV-I and -II peptides described herein, some cross-reactivity between the peptides is to be expected.

The format for the immunoassay in which peptides prepared according to the present invention are employed is dependent on whether antibody or antigen is to be determined. Immunoassay formats designed to detect antibody or antigen are well known in the art. Prior to or at the time of reacting the sample with the peptide, however, the chemical protection of the peptide is removed and the deprotected peptide is incubated under conditions conducive to disulfide bond formation. In assay formats where the peptide is immobilized to a solid phase the peptide cysteine residues may be deprotected in solution under conditions conducive to the formation of intramolecular disulfide bonds prior to or after immobilization.

The peptide compositions may be used unlabeled or labeled depending upon their application. (By label is intended a molecule which provides, directly or indirectly, a detectable signal.) Various labels may be employed, such as radionuclides, enzymes, fluorescers, chemiluminescers, enzyme substrates, cofactors or inhibitors, particles, e.g., magnetic particles, combinations of ligands and receptors, e.g., avidin and biotin, or the like. In addition, the polypeptides may be modified in a variety of ways for binding to a surface, e.g., microtiter plates, glass or latex beads, tubes, filters, chromatographic surfaces, e.g., paper, cellulose, silica gel, or the like. The particular manner in which peptides may be joined to another

compound or solid phase surface is conventional and finds ample illustration in the literature. See, for example, U.S. Patent Nos. 4,371,515; 4,487,715; and patents cited therein. Reagents such as p-maleimidobenzoic acid, p-methyldithiobenzoic acid, maleic acid anhydride, succinic acid anhydride, glutaraldehyde, hetero-bifunctional cross-linkers, and the like are commonly used for such purposes.

Various assay protocols may be employed for detecting the presence of either antibodies to retroviral proteins or retroviral proteins themselves. The peptide may be used as the labeled reagent, where the label allows for a detectable signal. Alternatively, the peptide may be immobilized either directly or indirectly on a surface, where antibody to the peptide in the sample will become bound to the peptide on the surface. The presence of human antibody bound to the peptide can then be detected by employing a xenogeneic antibody specific for human immunoglobulin, normally both human IgG and IgM, or a labeled protein specific for immune complexes, e.g., Rf factor or <u>S</u>. <u>aureus</u> protein A.

Various heterogeneous protocols may be employed, either competitive or non-competitive. Peptide may be bound to a surface or support and labeled antibody allowed to compete with antibody in the sample for the limited amount of bound peptide. The amount of label bound to the support would be related to the amount of competitive antibody in the sample.

Antibody could be bound to the support and the sample combined with labeled peptide. After contact of the reaction mixture with the bound antibody, the amount of label bound to the support would relate to the amount of cognate antibody in the sample.

Xenogeneic anti-human antibody, e.g., antibodies to the Fc of IgG and IgM (immunoglobulins), could be bound to a support. The sample would be contacted with the immunoglobulins and labeled peptide,

whereby the amount of labeled peptide bound to the support would be indicative of the presence of the cognate antibodies.

Illustrative of an assay technique is the use of immunoconcentration, e.g., filtration, where the subject peptide composition or conjugates thereof are adsorbed to a solid phase, e.g., latex beads, either covalently or noncovalently. The sample, a biological fluid sample, e.g., saliva, urine, cerebral spinal fluid, blood, plasma, or serum, may be pretreated by dilution into an assay medium, which will usually be an aqueous buffered medium employing one of a variety of buffers, such as phosphate, Tris, or the like, is combined with the antigen coated latex beads in a container, with a filter membrane, and a sufficient time allowed for complex formation between the peptide(s) and any cognate antibodies in the sample. The supernatant is allowed to pass through the filter and the filter upon which the coated beads are immobilized is washed to remove nonspecifically bound proteins.

A labeled specific binding protein which specifically binds to the complex is employed for detection. To the immunoreactive membrane may be added xenogeneic antisera to human immunoglobulin, particularly anti-(human IgG and IgM) in an appropriately buffered medium. The xenogeneic antisera will normally be labeled with a detectable label, e.g., enzyme or radionuclide. Instead of antisera, proteins specific for the immune complex may be employed, e.g., S. aureus protein A. The label may then be detected. For example, with an enzyme, after removal of non-specifically bound enzyme label, a developer solution is added. The developer solution will contain an enzyme substrate and possibly enzyme cofactors, chromogens, etc., which, upon reaction, provide a colored or fluorescent product which may be detected colorometrically or fluorimetrically, respectively.

The following examples are offered by way of
illustration and not by way of limitation..

EXAMPLE I
Thiol-Deprotected Peptides Detect
Antibodies to HIV-1 and HIV-2

Synthesis of Peptides

Derivatives of Peptide 39 were assembled by the
sequential coupling of t-butyloxycarbonyl-protected amino
acids onto 0.40 mmol p-methylbenzhydrylamine resin
(Applied Biosystems Inc., Foster City, CA). Amino acid
side chain protection was by standard benzyl based
groups. Tryptophan was protected by the formyl moiety.
When chemically reversible protection of a cysteine
residue was desired protection was provided by using
ethylcarbamoyl protection and coupling was achieved using
standard hydroxybenzotriazole ester chemistry as used for
glutamine or asparagine.

N-t-butyloxycarbonyl-S-ethyl-carbamoylcysteine
was prepared as described by St. Gutmann, and Storey et
al. supra. Briefly, 15.3 ml of trifluroacetic acid was
added dropwise into a mixture of 24.2g L-cysteine (free
base) and 200ml of dimethylformamide (DMF) at 0°C with
constant stirring. Ethyl isocyanate (17.8ml) was then
added dropwise and the mixture stirred at room
temperature for 18 hrs. The resultant mixture was
evaporated under vacuum and the residue dissolved in
180ml of water. Two portions of ether (100ml) cooled to
0°C was used to wash the aqueous solution and the pH was
adjusted to 7.0 by the addition of sodium hydroxide.
Absolute ethanol (550ml) was added, and the product was
allowed to crystallize overnight at 4°C. Washing the
crystalline solid with cold 20% ethanol/water and ether
after filtration yielded 18.9g of S-ethylcarbamoyl-
cysteine.

To make the N-t-butyloxycarbonyl derivative, 23g of solid di-t-butyl dicarbonate was slowly added to a vigorously stirring mixture of 18.9g S-ethylcarbamoyl-cysteine, 180ml dimethyl sulfoxide and 16.4ml diisopropylethylamine. The mixture was stirred for 2 hrs at room temperature before 400ml ice water plus 100ml of saturated sodium chloride was added. Hydrochloric acid was added to adjust the pH of the mixture to 2.0 and the aqueous mixture was washed with two, 200ml portions of ethyl acetate. The organic extracts were combined and evaporated under vacuum. The residue was recrystallized from ethyl acetate/hexane followed by recrystallization from 20% ethanol/water. A 19.4g yield of N-t-butyloxycarbonyl-S-ethylcarbamoylcysteine was obtained.

The final product was shown to be homogeneous by thin layer chromatography on silica gel with a chloroform/acetic acid (15:1) solvent; Rf 0.25. The melting point of the product was 144-146°C, similar to that previously reported to be 139-140°C (Storey supra).

Completed peptides were deprotected and cleaved from the resin by the standard high HF procedure or the low-high HF procedure of Tam et al. (J. Amer. Chem. Soc. 105:6442, 1983). Peptide was extracted from the resin in 50% acetic acid and subjected to gel chromatography in 20% acetic acid on G-25 Sephadex. Fractions containing peptide were pooled and lyophilized.

Immunological Reactivity

Peptide 39 and its analogues were tested for immunological reactivity by a test tube assay wherein the peptides were immobilized by adsorption or covalent bonding onto latex beads.

Adsorption of peptide 39GC and deprotected peptide 39GC onto latex beads was carried out by first washing the beads (5mg, IDC, Interfacial Dynamics Corporation, Portland Oregon, 1μm diameter) with

distilled water and resuspending in 0.5ml of 0.1M HEPES, pH 7.4, with vigorous mixing. Diluted peptide from a 4mg/ml 6M GuHCl stock solution was added to the washed beads (25-200μg peptide) to a final volume of 550μl. The mixture was vigorously stirred for 30 sec. followed by less vigorous mixing overnight. Following overnight incubation 500μl of distilled water was added and the beads were collected by centrifugation. The beads were washed with 1ml of distilled water twice before being suspended in 100μl of distilled water followed by the addition of 100μl 0.5N NaOH with immediate mixing to deprotect the cysteine residues of the adsorbed peptide. Deprotection was stopped with the addition of 1ml 0.25N HEPES, pH 7.4.

Air oxidation of the peptide/bead solution was carried out overnight at room temperature. Potassium ferricyanide was added to the peptide/bead solution to complete the oxidation. The tube was centrifuged and the beads were washed three times with 1ml of distilled water. Peptide/bead complex can be used immediately or stored as aqueous suspensions at 4°C for periods of up to 6 months without significant loss of reactivity.

Peptide 39, wherein X is -OH, Y is -NH$_2$ and cysteine thiol groups are not EC protected, was adsorbed to latex beads by adding 0.1ml of a 2mg/ml peptide stock solution of peptide 39 in 6M guanidine-HCl to washed latex beads (prepared as above) which had been resuspended in 0.9ml of 0.2M Tris, pH 8.0. The peptide latex bead suspension was incubated overnight at 45°C in a water bath. After incubation the beads were collected by centrifugation and washed once in 0.01M PBS, pH 7.0.

Peptide coated beads may be blocked, if necessary, to aid in the prevention of non-specific binding by the addition of 20μl of a 0.84% [w/v] suspension of coated beads to 1ml of 1% bovine serum albumin (BSA) and mixing vigorously. Beads were

collected by centrifugation and the supernatant removed. Wash steps were repeated twice subsequently.

Serum or plasma to be tested was diluted 1:80 in sample dilution buffer (2.5%[w/v] nonfat dry milk, 15ppm Kathon CG, 0.01% antifoam A in 0.01M sodium phosphate, pH 7.2, 0.15M sodium chloride) and 100μl was added to the blocked peptide/latex bead conjugate with mixing by vortex. The sample was incubated with the peptide/latex bead conjugate for 15 min. at room temperature before adding 0.9ml of wash solution (0.05% Tween 20 in Tris-saline, 1mM magnesium chloride), vortexing and centrifuging to collect the peptide/bead conjugate. Peptide/bead conjugate was washed again and collected by centrifugation and 100μl of a 1/100 dilution of anti human-IgG,A and M-alkaline phosphatase conjugate in dilution buffer was added for 15 min. at room temperature. The peptide/bead conjugate was washed again as above.

Immunological reactivity of the peptides was detected indirectly by the alkaline phosphatase activity. Enzyme buffer (0.8ml, 0.1M Tris-HCl/0.1M NaCl/5mM MgCl$_2$, pH 9.5) was added, followed by 100μl of substrate (9mg p-nitrophenyl phosphate in enzyme buffer). Substrate was incubated for 15 min. at 37°C and the reaction was stopped by the addition of 100μl of 3N sodium hydroxide. The beads were pelleted by centrifugation and the supernatant was measured for absorbance in a spectrophotometer at 405 nm. Peptides 39GC and 39GC deprotected showed substantial improvement over Peptide 39 in this assay format.

Alternatively, peptides were immobilized covalently onto latex beads through the carboxyl terminus. Carboxyl terminal conjugation was carried out with a peptide 39 analog, designated 39-I, which is comprised of the following sequence:

Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-
Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys*-
Ser-Gly-Lys-Leu-Ile-Cys*-Thr-Gly-SH

Cysteine residues were ethylcarbamoyl protected and the peptide was synthesized as described above. Completed peptide was cleaved from the solid phase using 90% hydrofluoric acid/10% anisole at 0°C, in vacuo, with stirring. Hydrofluoric acid was evaporated and the residue was washed with ethyl acetate prior to dissolving the crude peptide in 6M Gu-HCL/0.4M potassium phosphate. Crude peptide was citraconylated by the procedure of Blake et al. (Int. J. Peptide Res. 1981, 17:273, Proc. Natl. Acad. Sci. USA 1981, 78:4055, both incorporated herein by reference) followed by gel filtration in ammonium bicarbonate buffer. The resultant peptide, 39-IX, was comprised of an amino terminus and lysine residues which had been citraconylated, formyl-tryptophan, ethylcarbamoyl-cysteines and C-terminal thioglycine residue.

Peptide 39-IX was conjugated to the free amino groups of pentalysines conjugated to carboxylated latex beads (Seradyne, 1.1μm diameter, 0.01meq/g) via carbodiimide activation. An aliquot of bead suspension (0.3ml) was centrifuged to collect the beads. Beads were washed once with distilled water, collected and suspended in a solution of H-Lys-Lys-Lys-Lys-Lys-amide in water. The pH was adjusted to between 5.0 and 5.5 prior to the addition of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC). After standing overnight the beads were collected and washed with distilled water as above.

Half of the resultant beads were treated with 27μg/mg beads of peptide 39-IX in an aqueous solution of silver nitrate and 0.1M N-hydroxysuccinimide at pH 7.4 for 2 hrs. at room temperature. The beads were washed with 0.2M sodium cyanide and stored in 15% aqueous acetic acid overnight to remove the citraconyl groups.

Ethylcarbamoyl and formyl protecting groups were removed
by a brief treatment with 0.2N sodium hydroxide.
Cysteine residues were allowed to air oxidize overnight,
followed by reaction with potassium ferricyanide to
5   ensure complete oxidation.

The remaining pentalysine conjugated latex
beads were treated in an identical fashion as above,
except no silver nitrate was added. The resultant beads
served as a blank for comparison.

10   Peptide 39 derivatives were also covalently
immobilized through the amino terminus to carboxy
modified latex beads (Polysciences) via carbodiimide
activation. Eight milligrams of latex beads were placed
in a microfuge tube and 0.5ml of distilled water was
15   added. The beads were collected by centrifugation and
the supernatant removed. Activation was carried out by
adding 0.5ml of 0.2M EDC (38.2mg/ml) and 0.1M N-
hydroxysuccinimide (22mg/ml) and allowing the reaction to
proceed for 90 min. at room temperature with rotary
20   mixing. Activated beads were collected as above and all
but about 50μl of supernatant removed. The pellet was
dispersed and 250μl of 1M HEPES, pH 7.5, containing
various concentrations of peptide 39, were added (190 -
760μg). Peptide was allowed to react with the activated
25   beads overnight at room temperature with rotary mixing.
Beads were collected as above and washed with 0.1M HEPES,
pH 7.5. Two additional washes were carried out with 1%
BSA in Tris-saline(0.1M, pH 7.4) and the peptide/latex
beads were resuspended in 0.5 ml of 1% BSA/Tris-saline.
30   Alternatively N-terminal covalent
immobilization was carried out via thioether bond
formation. Peptide 39-GC was conjugated to NH_2 modified
latex beads (Pandex, Mundelein, IL). Five milligrams of
beads were washed once with distilled water and
35   resuspended in 200μl of 0.1M HEPES, pH 7.4.
N-succinimidyl-4-(N-maleimidomethyl)-cyclohexane-1-
carboxylate (SMCC, 100μl of 1mg/100μl in DMF) was added

to the bead suspension and allowed to react for 2 hrs. at
room temperature with constant mixing. Activated beads
were collected as above, washed twice with distilled
water, and once with 0.1M HEPES, pH 7.4, containing 5mM
EDTA. A 200μl suspension of activated beads in HEPES
buffer was prepared and 200μg of peptide 39-GC in 100μl
of 6M GuHCl was added. Conjugation was allowed to
proceed overnight at room temperature in a rotator.
Peptide conjugated beads were collected as above, washed
three times in distilled water and suspended at 0.84% in
distilled water. Immunologic cross-reactivity was tested
as described above.

Alternative methods of testing immunologic
cross-reactivity were also used. In one such method
Peptides 39 and 39GC were tested by ELISA as previously
described in US patent 4,629,783. Briefly, stock
solutions of peptides 39 and 39-GC were prepared in 6M
Gu-HCl. Working dilutions of 20μg/ml and 6μg/ml
respectively were prepared by dilution of stock solution
with 0.05M carbonate/bicarbonate buffer, pH 9.5. Wells
of microtiter plates were filled with 100μl of the
working dilution of the peptides and allowed to stand
overnight at room temperature. Coating solution was
removed and 300μl of blocking solution supra containing
0.5M ethanolamine was added for 1 hr. at room
temperature. Blocking solution was removed and the
plates were used immediately or allowed to dry and stored
for later use.

Plasma samples were diluted (See Table 1) in
sample dilution buffer (above) and 100μl was added to
individual wells. Samples were incubated for 30 min. at
37°C, then removed and the wells were washed six times
with 0.15M NaCl, 0.05% Tween 20. One hundred microliters
of goat antihuman Ig-horseradish peroxidase conjugate
diluted in citrate buffer, pH 7.0, 1% normal goat serum
was added to each well for 30 min at 37°C prior to
washing six times as above. The ELISA assay was

developed by adding 100μl/well of substrate solution (80μg/ml tetramethyl benzidine, 0.0015% hydrogen peroxide in citrate/phosphate buffer, pH 6.0) for 30 min. at room temperature.   Reactions were stopped with the addition of 100μl of 1N H_2SO_4 per well, and the ratio of the optical density at 450nm to 630nm was determined by an automated ELISA reader.   The cutoff value for a positive result was set at 0.225 Absorbance Units above the average absorbance obtained for three known negative samples. The results in Table 1 show that Peptide 39GC was able to give a positive result at a higher dilution than Peptide 39.

In separate experiments the thiol-protected peptide 39GC showed markedly reduced immunoreactivity in assays when compared to thiol-deprotected peptide 39GC. Similar results were observed with the HIV-2 peptide, 41-2-3GC.

TABLE 1
DILUTION PANEL COMPARISON OF PEPTIDE 39
AND PEPTIDE 39GC BY ELISA

Sample		Diagnosis 39GC/39	Confirmed Seropositive	PEPTIDE 39GC	PEPTIDE 39
PlasmaO	(1:40)	Pos/Pos	Yes	1.439	0.859
PlasmaO	(1:80)	Pos/Pos	Yes	1.010	0.547
PlasmaO	(1:160)	Pos/Pos	Yes	0.742	0.351
PlasmaO	(1:320)	Pos/Neg	Yes	0.397	0.197
PlasmaO	(1:640)	Neg/Neg	Yes	0.279	0.128
PlasmaO	(1:1280)	Neg/Neg	Yes	0.165	0.078
PlasmaG	(1:40)	Pos/Pos	Yes	0.895	0.585
PlasmaG	(1:80)	Pos/Neg	Yes	0.820	0.321
PlasmaG	(1:160)	Pos/Neg	Yes	0.575	0.241
PlasmaG	(1:320)	Neg/Neg	Yes	0.349	0.148
PlasmaG	(1:640)	Neg/Neg	Yes	0.179	0.092
PlasmaG	(1:1280)	Neg/Neg	Yes	0.107	0.078
Cutoff Value				0.348	0.341

EXAMPLE II
Membrane Concentration Immunoassay for
Antibodies to HIV-1 Using Deprotected Peptides

Various known serum and plasma samples were
screened for immunologic cross-reactivity using a
modified microparticulate assay employing the improved
methods and compositions of the present invention. The
subject peptide compositions were immobilized onto latex
beads and entrapped on a microporous membrane atop an
absorbent material for the determination and/or

quantitation of antibodies to HIV. Examples of such
immunoassay apparatus and methods are the subject of US
patents 4,633,901 and 4,727,019, both incorporated herein
by reference. The membrane concentration immunoassay
involves three incubations comprising a total assay time
of 10 minutes. Peptides tested were adsorbed to latex
beads by the methods described above.

Each sample was tested on a filtration unit
comprising three spots on a 1.2μm pore size nylon
membrane. One spot comprising the test peptide/latex
bead preparation, one spot comprising a positive
procedural control and the other comprising a negative
procedural control.

The peptide/latex bead preparation was
suspended in 0.1M Tris buffer, pH 7.2, 0.15M NaCl, 0.01M
MgCl$_2$, 10% sucrose, 2% fetal calf serum. Membranes were
spotted with a 0.1-0.2% suspension of peptide/bead
preparation. Negative procedural control spots were
comprised of latex beads coated by adsorption with BSA.
While positive procedural control spots were comprised of
latex beads coated by adsorption with goat antihuman
immunoglobulins.

Serum or plasma samples (40μl) were diluted
1:10 in dilution buffer (10% heat inactivated normal goat
serum, 1% dextran sulfate, 0.2% Tween 20, 2.5% nonfat dry
milk, 15ppm Kathon CG, 0.005% antifoam A, in 20mM sodium
citrate buffer, pH 7.4). Diluted specimen was added to
the surface of the membrane and allowed to absorb
completely. After waiting 2 min. from the time of
specimen addition, 1ml of wash buffer (0.01M Tris-
buffered saline, pH 6.0, 0.05M NaCl, 0.005M MgCl$_2$, 0.1%
sodium azide, 10μg/ml nitro blue tetrazolium, 0.5M
potassium thiocyanate) was added and allowed to
completely absorb through the membrane. About 0.150ml (3
drops) of goat anti-human Ig-alkaline phosphatase
conjugate diluted in 10mM phosphate buffered saline, pH
7.4, 0.2% goat gamma globulin, 1% bovine serum albumin,

1mM $MgCl_2$, 0.1mM $ZnCl_2$ was added to the membrane and allowed to absorb completely. The membrane was washed twice as above (2 ml) and about 0.150ml (3 drops) of substrate (1M 2-amino-2-methyl-1-propanol, pH 10.3, 5mg/ml 3-indoxyl phosphate) was added and allowed to absorb completely. After about 5 min. about 1ml of 0.5M EDTA, pH 7.4, was passed through the membrane to stop the reaction. After the stop solution was completely absorbed the units were read visually or semi-quantitatively by reflectance.

A result was negative if only the positive control spot showed any sign of color development and the test spot and the negative control spot showed no color development. A positive result was indicated if the test spot showed some level of color development (trace to 4+), the positive control spot shows color development and the negative control spot showed no color development. The test was considered to be invalid if all spots showed any color development, no color development was seen for the positive control, or if the negative control spot showed any color development.

A wide variety of positive, negative, EIA positive/western blot atypical and immune sensitized specimens have been tested with the preferred peptide 39 analog, peptide 39-GC, as the test antigen. Positive specimens showed a gradation of reactivity from 0.5+ to 4+, all valid tests resulting in clearly defined colored spots on a clean white background. The negative control showed no color development and the positive control gave approximately 2+ coloration.

Table 2

SUMMARY OF RESULTS OF VARIOUS TYPES OF SAMPLES
TESTED BY MEMBRANE IMMUNOCONCENTRATION IMMUNOASSAY
WITH PEPTIDE 39-GC AS THE ANTIGEN

Category	No. Positive/Total No.
Normal Blood Bank Donors	0/1187
Western Blot Neg./LAV-EIA Pos.	0/79
Western Blot p25 Neg./LAV/EIA Pos.(Atypical 1)	0/39
Western Blot p25 Pos./LAV-EIA Pos.(Atypical 2)	0/170
Western Blot Pos./LAV-EIA Pos.(OD<1.5)	120/120
Western Blot Pos./LAV-EIA Pos.(OD>1.5)	29/29
Autoimmune Samples1	0/51
Secondary, Atypical Syphillis Sera/HIV Neg.	0/26
Secondary, Atypical Syphillis Sera/HIV Pos.	4/4
Problematic HIV-I Seronegative Samples2	0/72

n=1777

1. Autoimmune samples included 11 asthma patients, 11
Sjogren's syndrome patients, 7 systemic lupus erythematosus
patients, 4 ankylosing spondylitis patients, 3 unknown
conditions and 15 assorted syndromes/diseases.
2. Icteric, lipemic, bacteremic, ascorbic acid and various
anticoagulant treated samples.

Various peptide compositions of the subject
invention have been tested using some of the assay
formats described above. A summary of the results is
found in the following tables. In Table 3 results of the
comparison of Peptide 39 by ELISA and membrane
concentration immunoassay with whole virus ELISA on
atypical samples are given. Ten of the eleven atypical
samples tested positive by whole virus ELISA and were not

confirmed to be positive by Western Blot. All were found
to be negative when Peptide 39 was used as the
immobilized antigen by both ELISA and membrane
concentration immunoassay.

Results of whole virus ELISA with Peptide 39GC
by ELISA and membrane concentration immunoassay and
Peptide 39 by ELISA on two seroconversion panels are
presented in Table 4. Peptide 39GC and peptide 39 gave a
positive result earlier than whole virus ELISA. Peptide
39GC gave a positive result earlier than peptide 39 on
one of the seroconversion panels.

TABLE 3

ENZYME LINKED IMMUNOASSAY COMPARISON OF PEPTIDE AND WHOLE VIRUS AS ANTIGEN ON WESTERN BLOT ATYPICAL SERUM SAMPLES

Specimen	Diagnosis LAV/Peptides	Confirmed Seropositive	LAV ELISA	Peptide 39 ELISA	Peptide 39 MCI[1]
Y34150	Pos/Neg	No	0.526	0.063	Neg
Z00132	Neg/Neg	No	0.307	0.083	Neg
H62111	Pos/Neg	No	0.751	0.047	Neg
V45719	Pos/Neg	No	0.581	0.048	Neg
Y49300	Pos/Neg	No	0.523	0.048	Neg
V44932	Pos/Neg	No	0.617	0.068	Neg
G16211	Pos/Neg	No	0.387	0.027	Neg
G15694	Pos/Neg	No	0.367	0.078	Neg
Y40646	Pos/Neg	No	0.655	0.057	Neg
H63547	Pos/Neg	No	0.901	0.092	Neg
Y38248	Pos/Neg	No	0.386	0.048	Neg

[1]Membrane Concentration Immunoassay

TABLE 4
COMPARATIVE RESULTS USING PEPTIDE AS ANTIGEN
IN ELISA AND MEMBRANE CONCENTRATION IMMUNOASSAY
FORMATS FOR TWO SEROCONVERSION PANELS

Sample Number	Diagnosis	Confirmed Seropositive	LAV ELISA	Peptide 39GC	Peptide 39	MCI 39GC
BBI-1	Negative	No	0.067	0.347	0.128	-
BBI-2	Negative	No	0.075	0.317	0.129	-
BBI-3	Pos/Neg	Yes	0.180	0.842	0.514	+
BBI-4	Positive	Yes	0.858	1.105	0.632	++
BBI-5	Positive	Yes	1.164	1.757	1.098	++
BBI-6	Positive	Yes	1.359	2.160	1.531	++
BBI-7	Positive	Yes	1.430	1.986	1.307	++
BBI-8	Positive	Yes	1.556	2.317	1.688	+++
BBI-9	Positive	Yes	1.507	2.328	2.122	+++
Cutoff Value			0.291	0.384	0.341	
BBI-20	Negative	No	0.038	0.136	0.037	-
BBI-21	Negative	No	0.055	0.158	0.053	-
BBI-22	Pos/Neg	Yes	0.057	0.450	0.244	+
BBI-23	Pos/Neg	Yes	0.123	0.890	0.540	+
BBI-24	Pos/Neg	Yes	0.127	1.064	0.636	+
BBI-25	Pos/Neg	Yes	0.216	0.845	0.553	++
BBI-26	Pos/Neg	Yes	0.259	0.852	0.445	++
BBI-27	Positive	Yes	0.335	1.104	0.590	++
BBI-28	Positive	Yes	0.431	1.177	0.769	++
BBI-29	Positive	Yes	0.718	2.085	1.566	++
BBI-30	Positive	Yes	0.837	2.214	1.871	+++
BBI-31	Positive	Yes	0.798	2.207	1.812	+++
BBI-32	Positive	Yes	0.920	2.263	1.925	+++
BBI-33	Positive	Yes	0.833	2.277	1.961	+++
BBI-34	Positive	Yes	0.728	1.622	1.363	+++
BBI-35	Positive	Yes	0.902	2.198	1.861	+++
BBI-36	Positive	Yes	0.881	2.265	1.959	+++
BBI-37	Positive	Yes	0.953	2.105	1.530	+++
Cutoff Value			0.291	0.384	0.341	

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EXAMPLE III

Detection of Antibodies to HIV-2 Using Deprotected Peptides

The immunoreactivity of HIV-2 peptides synthesized
with protected cysteine thiol groups was determined. HIV-2
peptide 41-2-3 was synthesized as described in Example I, with
the Cys groups protected and having an N-terminal Cys-Gly-Gly,
yielding peptide 41-2-3GC. EIAs were performed as described
above using viral lysates of HIV-1 and HIV-2 and peptides 39GC
(HIV-1) and 41-2-3GC. Reactivity was determined for dilutions
of two HIV-2 positive sera, F504282, obtained from a patient in
New York, and "GOM," provided by the Institut Pasteur. The
results, shown in Table 5, demonstrate that the synthetic
peptides clearly differentiate between HIV-1 and HIV-2 and
provide particularly enhanced specific immunoreactivity when
compared to results obtained with the whole virus lysate EIAs.

Table 5:   Reactivities in EIA of dilutional series prepared
           from HIV-2 positive specimens

Dilution	HIV-1 Peptide 39GC	HIV-2 Peptide 41-2-3GC	HIV-1 Lysate	HIV-2 Lysate
A*				
Neat	0.075	3.000	ND#	2.026
1:5	0.049	3.000	0.494	1.808
1:10	0.042	3.000	0.293§	1.687
1:20	0.037	3.000	0.170	1.405
1:40	0.035	2.828	0.101	1.182
1:80	0.033	2.107	0.072	0.876
1:160	0.040	1.441	0.046	0.597
1:320	0.031	0.941	0.039	0.367
1:640	0.031	0.588	0.034	0.171
1:1280	0.039	0.302	ND	ND
1:2560	0.030	0.180	ND	ND
- -				
B*				
1:2	0.052	3.000	0.839	2.113
1:4	0.037	3.000	0.510	1.953
1:8	0.040	3.000	0.317	1.703
1:16	0.031	3.000	0.177	1.433
1:32	0.035	3.000	0.099	1.083
1:64	0.074	2.524	0.060	0.736
1:128	0.031	2.202	0.047	0.552
1:256	0.035	1.452	ND	0.280
1:512	0.040	0.811	ND	0.176
1:1024	0.037	0.571	ND	0.114

* A is serum from patient F504282, B is serum from patient
    GOM.
ND= Not Determined.
§ Line represents cutoff, values above which are positive and
    below are considered negative.

         The immunoreactivity of unprotected HIV-2 peptide
41-2-3 was compared to the deprotected form having a Cys-Gly-
Gly extension at the N-terminus (41-2-3GC), and with an HIV-2
whole virus lysate. The assays were EIAs performed generally
as described above with varying dilutions of HIV-2 positive
serum specimens. The results for representative sera are
shown in Table 6.

Table 6: Reactivity of Selected HIV-2 Sera with Deprotected and Unprotected HIV-2 Peptides and HIV-2 Viral Lysate.

Specimen	Dilution	41-2-3GC	41-2-3	Lysate
"GOM"	1:2	3.000	2.239	2.113
	1:4	3.000	1.932	1.953
	1:8	3.000	1.517	1.703
	1:16	3.000	1.143	1.433
	1:32	3.000	0.693	1.083
	1:64	2.524	0.343[§]	0.736
	1:128	2.202	0.228	0.552
	1:256	1.452	0.092	0.280
	1:512	0.811	0.069	0.176
	1:1024	0.571	0.058	0.114
"PIN"	1:2	3.000	2.082	1.336
	1:4	3.000	1.695	1.035
	1:8	2.596	1.154	0.834
	1:16	1.940	0.607	0.711
	1:32	1.209	0.369	0.387
	1:64	0.582	0.182	0.188
	1:128	0.368	0.123	0.147
	1:256	0.186	0.091	0.087
"SAMB"	1:2	3.000	1.535	1.047
	1:4	3.000	1.089	0.968
	1:8	2.854	0.659	0.708
	1:16	2.710	0.433	0.485
	1:32	2.112	0.239	0.398
	1:64	1.400	0.121	0.289
	1:128	0.834	0.081	0.144
	1:256	0.404	ND[#]	ND
"S"	1:2	3.000	2.208	1.855
	1:4	3.000	1.942	1.806
	1:8	3.000	1.440	1.590
	1:16	3.000	0.910	0.917
	1:32	2.632	0.581	0.647
	1:64	2.155	0.273	0.455
	1:128	1.442	0.159	0.289
	1:256	0.725	0.089	0.157
	1:512	0.516	0.061	0.103

[#] ND= Not Determined.
[§] Line represents cutoff, values above which are positive and below are considered negative.

EXAMPLE IV
Detection of Antibodies To HTLV-I Using Deprotected Peptides

A region of HTLV-I transmembrane glycoprotein, gp21, was identified as corresponding to transmembrane regions of HIV-1 and HIV-2 and thus potentially immunodominant. Synthetic peptides were prepared based on the known amino acid sequence of HTLV-I. Peptides were synthesized and protected generally according to the procedures outlined in Example I. Assays were performed generally as described in Examples I and II. The results are shown in Table 6.

Table 6: HTLV-I Peptides Tested for Reactivity to Antibodies to HTLV-I.

Peptide[§] /Sequence	Serum Sample[#]		
	40359	0435	032EC1
121-1G1			
H- QNRRGLDLLFWEQGGLC*KALQEQC*-NH$_2$	trace	-	-
121-1GC1			
H- CGG-QNRRGLDLLFWEQGGLC*KALQEQC*-NH$_2$	3+	1+	-
121-2G1			
H- GLDLLFWEQGGLC*KALQEQC*-NH$_2$	-	-	-
121-2GC1			
H- CGG-GLDLLFWEQGGLC*KALQEQC*-NH$_2$	3+	0.5+	-
121-3G1			
H- NRRGLDLLFWEQGGLC*-NH$_2$	-	-	-
121-3GC1			
H- CGG-NRRGLDLLFWEQGGLC*-NH$_2$	3+	-	-
121-3EG1			
H- C*GG-NRRGLDLLFWEQGGLC*-NH$_2$	ND	-	-
121-1D1			
H-QNRRGLDLLFWEQGGLCKALQEQC-OH	ND	-	-

§ - Coating level for peptides on latex microparticles was 50 μg peptide/ 5 mg beads/ 550 μl volume.

- Serum samples 40359 and 0435 are HTLV-I EIA positive control sera used undiluted; 032EC1 is HTLV-1 EIA Negative control.

* - Indicates Cys residue was protected during synthesis using ethylcarbamoyl protection.

Results of assays with peptide 121-1GC1 were compared to those obtained with other HTLV-I/II tests,

such as the polymerase chain reaction (PCR), radioimmunoprecipitation (RIP), Western blot (WB), and viral lysate EIAs. The procedure as described in Example II was used for peptide 121-1GC1, except that two drops of samples were used and the conjugate was used at a two-fold higher concentration. The assays were performed with 10 serum samples suspected of containing antibodies to HTLV-I and/or HTLV-II. The PCR assay for HTLV-II was performed generally according to PCR Technology, Principles and Applications for DNA Amplification, ed. H.A. Ehrlich, Stockton Press, N.Y. (1989), incorporated herein by reference. The results are shown in Table 7.

TABLE 7: Comparison of Assays for HTLV-I/II with Deprotected Peptide 121-1GC1.

Sample No.	PCR HTLV-II	EIA HTLV-I	RIP HTLV-1	WB HTLV-1	Status	121-1GC1
89379	+	+	+	+	+	trace
89365	+	+	-	vw[§]	Ind[#]	0.5+
39266	+	+	-	vw	Ind	trace
89430	+	+	-	+	Ind	trace
89433	+	+	-	-	Ind	1+
89442	-	+	-	Ind	Ind	trace
89232	+	+	+	+	+	trace
89283	+	+	+	+	+	1+
89359	+	+	+	+	+	1+
88002					-	-
12-97-854					-	-

Coating Level: 80_g peptide/5mg bead/550_l volume
[§] - vw is very weak reaction.
[#] - Ind is indeterminant status.

In other experiments, screening of the HTLV-I peptide in a variety of forms against a serum panel of suspected HTLV-I/II positive individuals yielded inconclusive results when compared to results obtained by RIP, Western blot, and commercially available HTLV-I EIA assays. Some samples which were purportedly positive by certain of the tests were negative with peptide 121-1GC1. These results may be due to several factors, including

the possibility that samples negative by peptide 121-1GC1 and positive by other criteria contained antibodies predominantly reactive with virus core antigens and not with transmembrane glycoprotein antigens.

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.